SLU 4554.3
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Minteer, et al. Art Unit 1745
Serial No. 10/617,452
Filed July 11, 2003
Confirmation No. 4859
For ENZYME IMMOBILIZATION FOR USE IN BIOFUEL CELLS AND SENSORS
Examiner Angela J. Martin

DECLARATION OF SHELLEY D. MINTEER UNDER 37 CFR 1.132

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS,

SIR:

I, Shelley D. Minteer, hereby declare and state as follows:

1. I reside at 2150 Gregory, Pacific, Missouri 63069.

2. I received a Doctor of Philosophy in Chemistry from the University of Iowa in 2000.

3. I am currently an Associate Professor of Chemistry at Saint Louis University in St. Louis, Missouri.

4. I am a co-inventor of the subject application, which claims bioanodes and biofuel cells comprising bioanodes.

5. I have reviewed the Office action dated December 10, 2007 in the subject application.

6. I am providing this Declaration to address whether glucose oxidase is stabilized by cocasting with unmodified Nafion® as would be required for the Nafion® to be an

enzyme immobilization material that immobilizes and stabilizes the enzyme as required by claim 6.

7. Glucose oxidase (GOx) catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone with the concurrent release of hydrogen peroxide. It is highly specific for β-D-glucose and does not act on α-D-glucose. In the presence of peroxidase, hydrogen peroxide enters into a second reaction in the assay involving p-hydroxybenzoic acid and 4-amino antipyrine with the quantitative formation of quinoneimine dye complex, which can be measured at 510 nm.

8. Under my direction and control, the activity of glucose oxidase (GOx) enzyme was measured in each of the hydrophobically modified Nafion® membranes. The hydrophobically modified Nafion® membranes were prepared by casting a suspension of Nafion® polymer with a solution of a salt of a hydrophobic cation such as quaternary ammonium bromide. Excess quaternary ammonium bromide or hydrogen bromide were removed from the membrane before it was re-cast to form the salt-extracted membrane. The immobilized GOx enzyme was then cast in a plastic vial and the absorbance was measured at 510 nm against water. All experiments were performed in triplicate and reported uncertainties correspond to one standard deviation.

9. Attachment A of this declaration shows a graph of the glucose oxidase activity in buffer and when immobilized in various Nafion® membranes, including an unmodified Nafion® membrane. Various quaternary ammonium cations used were trimethyloctyldecylammonium (TMODA), trimethylhexyldecylammonium (TMHDA), trimethyltetradecylammonium (TMTDA), trimethyloctylammonium (TMOA), trimethyldodecylammonium (TMDDA), trimethyldecylammonium (TMDA), trimethylhexylammonium (TMHA), tetrabutylammonium (TBAB), and triethylhexylammonium (TEHA). This graph shows that the activity of the glucose oxidase is greatly reduced when cocast in an unmodified Nafion® membrane as compared to glucose oxidase in a buffer. Also, glucose oxidase activity increased when

immobilized in various modified Nafion® membranes as compared to the glucose oxidase in buffer.

10. I hereby declare and state that all statements made herein are to my own knowledge true; and that all statements made on information and beliefs are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements will jeopardize the validity of the above-identified application or any patent issued thereon.

Shelley D. Minteer

March 7, 2008

Shelley D. Minteer Date

Attachment A